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13. ABSTRACT (Maximum 200 words) We have, to date, 57 Hereditary Breast/Ovarian Cancer families with an identified BRCA1 or BRCA2 genetic mutation, wherein about 107 breast cancer patients' slides and tissue blocks have been retrieved together with medical histories, pedigree relationships, survival data, and 115 completed risk factor questionnaires. Previous findings have shown that <i>BRCA1</i> -related and other HBC patients presented at lower stage ($p = 0.003$) and at an earlier age than non-HBC patients (mean, 42.8 years and 47.1 years vs. 62.0 years, $p < 0.0001$). Basic pathology findings indicated that when compared with non-HBC, invasive <i>BRCA1</i> -related HBC had a statistically significantly lower diploidy rate, a lower mean aneuploid DNA index, and a strikingly higher proliferation rate. Among aneuploid tumors, S-phase fraction were higher in the <i>BRCA1</i> mutation carriers. <i>BRCA2</i> patients had more tubular-lobular group carcinomas ($OR = 2.56$, $p = 0.007$). All these findings were independent of age. We are now in the process of collecting, for examination, environmental and known endogenous risk factors, which will be compared with a control group employing multiple regression analysis. Knowledge from this research will have a significant translational impact upon more common sporadic reoccurring breast cancer. Long-term, this may even impact upon DNA-targeted chemotherapy.				
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FOREWORD

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TABLE OF CONTENTS

	Page numbers
Front Cover	1
Report Documentation	2
Foreword	3
Table of Contents	4
Introduction	5
Body	5
Key Research Accomplishments	8
Reportable Outcomes	8
Conclusions	8
References	11
Appendices	13

INTRODUCTION

Despite significant advances in chemotherapy, radiation therapy and surgery, the survival rate of patients with breast cancer has shown only marginal improvement during the past several decades. Our purpose is to extend our investigation to approximately 100 families with approximately 400 breast cancer patients where slides and tissue blocks will be retrieved and medical histories including risk factors and survival parameters will be documented. We postulate that mutations within BRCA1 and BRCA2 might differentially predispose breast cancer patients to specific phenotypic changes, such as differences in pathobiology and survival. These concerns are clearly cogent to a better understanding of the etiology, pathogenesis, and survival of breast cancer affected patients from BRCA1 and BRCA2 mutation positive families. This knowledge could ultimately impact upon breast cancer in the general population.

BODY

Fifty-seven Hereditary Breast/Ovarian Cancer families have been ascertained for this study to date. These families were initially classified as HBC or HBOC (Hereditary Breast Ovarian Cancer) because breast and certain site specific cancers (particularly ovarian) occurred in a pattern consistent with a highly penetrant, autosomal dominant disorder, of which certain cardinal features are early ages at cancer onset and multiple primary and/or bilateral breast cancer diagnoses¹. Two hundred and twenty-eight subjects were eligible for this study from these families where gene linkage studies and/or germline mutations for BRCA1 and BRCA2 have been performed. Eight of these cases were part of the preliminary study done in 1996, but were not completed at that time.

All living subjects and the next of kin of deceased subjects were asked to sign an informed consent form to release clinical information, slides, and tissue blocks. To date, 174 informed consents have been returned. Of the remaining 54 individuals, 38 have not responded to the request, 8 individuals have been lost to contact, 5 individuals were found to be deceased, and 3 individuals did not wish to participate.

In addition to the informed consent, a risk factor questionnaire was mailed out to all living subjects for completion. In the first year, 102 questionnaires were filled out and returned and 13 more were completed over the second year. These questionnaires have been forwarded to Dr. Steven Narod for data entry and assessment of survival parameters in context with their staging classification.

Upon receipt of the signed consent form, requests for slides, tissue blocks, and clinical information of the subjects' breast cancer diagnoses were sent to the appropriate hospitals. Of the 174 requests, 35 hospitals did not respond to the initial request (subsequent requests were not answered also). Thirty-three institutions reported the slides and blocks to be destroyed, missing, or not available. Unfortunately, the main reason given by the hospitals and laboratories for not having the samples was that their policy dictated destruction of samples after a certain period of time. We will continue the search in order to achieve 400 samples over the time of the grant.

We have ascertained, over the past year, 107 case samples of slides and/or blocks. Once slides and blocks were received, a random number was assigned and the H&E stained slides and corresponding blocks were sent to Dr. John Bishop in the Department of Pathology at Creighton

University for selection of adequate specimens. Once slides were selected, additional ones were cut from the accompanying blocks. Quantitative DNA flow cytometry was then performed on the blocks by Dr. Bishop. The DNA histograms will be classified and scored with regard to ploidy, DNA index and S-phase fraction, as described².

H&E slides have been sent to Joseph Marcus, MD and David Page, MD for histopathological classification and grading. The two pathologists simultaneously will view the slides in a two-headed microscope and arrive at a consensus diagnosis using the WHO breast cancer classification³ with minor modifications⁴. The status of the slides and blocks, to date, is presented in Table 1.

Upon completion of the analysis and review of the slides and blocks and risk factor questionnaires, significances of differences between BRCA1 and BRCA2, and among mutation-defined subgroups of BRCA1 and BRCA2, will be assessed by chi-square or Fisher's exact test or one-way ANOVA (for frequencies) and the Mann-Whitney U test (for means of continuous variables). Logistic regression will be used to assess the effects of patient age and other co-variates on data trends.⁵ Odds ratios with 95% confidence intervals (CI) will be calculated for pathobiologic traits for BRCA1 vs BRCA2 differences.

Differences in time to death (crude death rate), time to first recurrence (recurrence rate), and time to breast cancer death (breast cancer-specific death rate) will be assessed by Cox proportional hazards survival analysis⁶, using EGRET software (Statistics and Epidemiology Research Corporation, Seattle, WA). Hazards ratios (HR) of BRCA1 vs. BRCA2, with 95% confidence

intervals, will be calculated for regression terms. S. Narod, M.D., will perform risk factor analysis and will assist with survival analysis.

For the start of the third year, at least 6 new families have a newly identified BRCA1 or BRCA2 mutation and will be included in the study. We look forward to, throughout the upcoming year, the identification of new families for this study from Dr. Narod's Laboratory.

KEY RESEARCH ACCOMPLISHMENTS & REPORTABLE OUTCOMES

- Tissue slide and block collection of 107 cases.
- Database of 115 completed risk factor questionnaires at Dr. Steven Narod's office.

CONCLUSIONS

Novel research studies are needed to better comprehend the etiology and pathobiology of breast cancer in the hope of translation to therapeutic benefit. BRCA1 and BRCA2 families provide extraordinary research models to test hypotheses relevant to the pathobiology and survival of breast cancer. Might BRCA2 cancers be more sensitive to radiation?⁷ Are known breast cancer risk factors important in BRCA1 and BRCA2? Are there survival differences in BRCA1 and BRCA2 breast and ovarian cancers? To date, this risk factor information has provided only partial explanations as to their impact at the molecular genetic level.

Some evidence has shown differences in the pathobiology, and prognosis in harbingers of BRCA1 and BRCA2 mutations.² Specifically, BRCA1-related and other HBC patients both

presented at lower stage ($p = 0.003$) and earlier age than non-HBC patients (mean, 42.8 years and 47.1 years vs. 62.0 years, $p < 0.0001$). Compared with non-HBC, invasive BRCA1-related HBC had a lower diploidy rate (13% vs. 35%; $p = 0.002$), lower mean aneuploid DNA index (1.53 vs. 1.73; $p = 0.002$), and strikingly higher proliferation rates (mitotic grade 3; odds ratio [OR] = 4.42; $p = 0.001$; aneuploid mean S-phase fraction 16.5% vs. 9.3%, $p < 0.0001$). Other HBC patients, including patients in two BRCA2-linked families, had more tubular-lobular group (TLG) carcinomas (OR = 2.56, $p = 0.007$). All trends were independent of age. A nonsignificant trend toward better crude survival in both HBC groups was age- and stage-dependent. Compared with non-HBC in age and stage adjusted analysis, BRCA1-related HBC patients survived no longer. Other HBC patients, despite neutral prognostic indicators, fared worse. Subsequently, a subset analysis based exclusively on BRCA1 and BRCA2 germline mutations clearly defined the pathobiology separation between BRCA1 and BRCA2.^{8,9,10}

To date, of the original 228 individuals targeted from the 57 HBOC families, we have been able to acquire 107 cases of slides and/or blocks. Dr. Marcus and Dr. Page are currently reviewing 54 of these cases and Dr. Bishop is currently working on 12 cases with 23 cases already finished with DNA flow cytometry (See Table 1). One hundred and fifteen risk factor questionnaires have been completed over the course of the grant. Once there has been a sufficient amount of cases completed, analysis of etiology, pathogenesis, and survival of these breast cancer affected patients will be started. Mutations within the BRCA1 and BRCA2 genes will be utilized as part of a subset analysis in an attempt to correlate phenotype with specific genotype. When analysis is completed, we will know whether mutations within BRCA1 and BRCA2 might predispose breast cancer patients to specific phenotypic changes. This could be important in the future for

the development of gene therapy, particularly when considering potential differences in the phenotypic effects of mutations within BRCA1 and BRCA2.

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Table 1: Statutes of Individuals' Cases of blocks and slides
 "Hereditary Breast Cancer: Mutations Within BRCA1 and BRCA2 with Phenotypic Responses."

BLOCKS

	Blocks destroyed/ missing/ not avail. from hospital	Blocks to be requested after slides finished	Blocks pending DNA Flow	DNA insufficient for Flow Cytometry	Blocks currently being run by Dr. Bishop	Blocks finished with DNA Flow Cytometry
No response from indiv./kin regarding request for slides						1
No response from hospital regarding request for slides					1	
Slides destroyed/ missing/ not avail. from hospital						2
Slides to be requested after blocks finished			7			2
Slides pending review by Dr. Marcus & Dr. Page	5	8	3		8	8
Slides currently being reviewed by Dr. Marcus & Dr. Page	8	15	17	1	3	10

SLIDES